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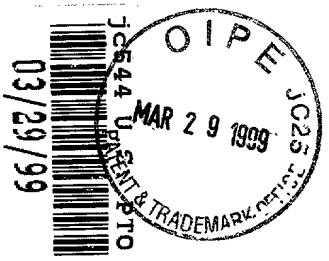

Patent
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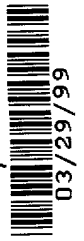

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Anticipated Classification of this application:
Class ____ Subclass ____
Prior application:
Examiner:
Art Unit:

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D. C. 20231

FILING UNDER 37 CFR § 1.53(B)

This is a request for filing for a

☒ continuation ☐ divisional ☐ continuation-in-part (CIP)

application under 37 CFR § 1.53(b) of pending prior application Serial No.. 08/737,446 filed on May 12, 1995 (international application filing date is May 12, 1995; but date on filing receipt is January 10, 1997),

of: John Dupre

for: TREATMENT OF DIABETES

I. COPY OF PRIOR APPLICATION AS FILED WHICH IS ATTACHED

☒ I hereby verify that the attached papers are a true and complete copy of what is shown in my records to be the above-identified prior application, including the oath or declaration as originally filed. (37 CFR § 1.53)

14 Page(s) of Specification
3 Page(s) of Claims
Page(s) of Abstract

SD-109179.1

CERTIFICATE OF MAILING
(37 C.F.R. §1.10)

I hereby certify that this paper (along with any referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as 'Express Mail Post Office To Addressee' in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

EL088408052US
Express Mail Label No.

March 29, 1999
Date of Deposit

Irene Grimes Betke
Name of Person Mailing Paper

Irene Grimes Betke
Signature of Person Mailing Paper

10 Sheet(s) of Drawings _____ formal X Informal
2 Page(s) of Declaration and Power of Attorney
_____ Small Entity Statement

☒ Pursuant to 37 CFR 1.63(d)(1), a newly executed oath or declaration is not required.

☐ A newly executed oath or declaration is filed herewith

☐ I hereby state that the amendment referred to in the declaration filed to complete the prior application, in accordance with the requirements of 37 CFR § 1.53(b), did not introduce new matter therein.

II. AMENDMENTS

☐ Cancel in this application original Claims _____ of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

☒ A Preliminary Amendment is enclosed. (Claims added by Amendment must be numbered consecutively beginning with the number next following the highest numbered original claim in the prior application.)

III. INFORMATION DISCLOSURE STATEMENT

☐ An Information Disclosure Statement, PTO 1449, and references are submitted herewith.

IV. PETITION FOR SUSPENSION OF PROSECUTION FOR THE TIME TO FILE AN AMENDMENT

☐ There is provided herewith a PETITION FOR SUSPENSION OF PROSECUTION FOR THE TIME NECESSARY TO FILE AN AMENDMENT (NEW APPLICATION FILED CONCURRENTLY).

V. FEE CALCULATION

BASIC FILING FEE:							$760.00
Total Claims	20	-	20	=	0	x $18.00	$0.00
Independent Claims	4	-	3	=	1	x $78.00	$78.00
Multiple Dependent Claims	$260	(if applicable)				☒	$260.00
Surcharge 37 CFR § 1.16(e)	$130	(if applicable)				☐	$0.00
TOTAL OF ABOVE CALCULATIONS							$1,098.00

Reduction by ½ for Filing by Small Entity. Note 37 CFR §§ 1.9, 1.27, 1.28. If applicable, Verified Statement must be attached. ☒	$549.00
Misc. Filing Fees (Recordation of Assignment)	$0.00
TOTAL FEES SUBMITTED HEREWITH	$549.00

☐ The fee for extra claims is not being paid at this time.

VI. SMALL ENTITY STATUS

A Verified Statement to establish small entity under 37 CFR §§ 1.9 and 1.27:

☐ is attached

☒ has been filed in the prior application and such status is still proper and desired. [37 CFR § 1.28(a)]

Filing Fee Calculation (50% of above) $510.00

VII. DRAWINGS

[NOTE: DO NOT CHECK THIS IF PRIOR CASE IS NOT TO BE ABANDONED.]

☐ Transfer the drawings from the prior application to this application and, subject to Item 16 below, abandon said prior application as of the filing date accorded to this application. A duplicate copy of this request is enclosed for filing in the prior application file.
[May only be used if signed by (1) applicant, (2) assignee of record or (3) attorney or agent of record and before payment of issue fee. 37 CFR § 1.138.]

☐ Transfer the following sheet(s) of drawings from the prior application to this application.

☒ New drawings are enclosed ☐ formal ☒ informal

VIII. PRIORITY - 35 USC § 119

☐ Priority of application Serial No. _____ filed on _____ in _____ is claimed under 35 USC § 119.

☐ The certified copy has been filed in prior U.S. application Serial No. _____on _____.

☐ The certified copy will follow.

IX. RELATE BACK - 35 USC § 120

☒ Amend the Specification by inserting before the first line the sentence:
This is a continuation of co-pending application Serial No. 08/737,446 filed May 12, 1995.

X. INVENTORSHIP STATEMENT

☒ With respect to the prior co-pending U.S. application from which this application claims benefit under 35 USC § 120, the inventor(s) in this application is (are):

☒ the same

☐ less than those named in the prior application and it is requested that the following inventor(s) identified above for the prior application be deleted:

[Name(s) of inventor(s) to be deleted]

☒ The inventorship for all the claims in this application are:

☒ the same

☐ not the same, and an explanation, including the ownership of the various claims at the time the last claimed invention was made, is submitted.

XI. ASSIGNMENT

☒ The prior application is assigned of record to Amylin Pharmaceuticals, Inc..

☐ An Assignment of the invention to _____ is attached.

XII. FEE PAYMENT BEING MADE AT THIS TIME

☐ Not attached. No filing fee is submitted. [This and the surcharge required by 37 CFR § 1.16(e) can be paid subsequently.]

☒ Attached.

☒	Filing fees.	$549.00
☐	Recording assignment. [**$40.00** 37 CFR § 1.21(h)(1)]	------
	Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached. [**$130.00**; 37 CFR §§ 1.47 and 1.17(h)]	

☐	Petition fee to Suspend Prosecution for the Time Necessary to File an Amendment (New Application Filed Concurrently.) [**$130.00**; 37 CFR §§ 1.103 and 1.17(i)]	------
☐	For processing an application with a specification in a non-English language. [**$130.00**; 37 CFR §§ 1.52(d) and 1.17(k)]	------
☐	Processing and retention fee. [**$130.00**; 37 CFR §§ 1.53(f) and 1.21(l)]	------
	Total Fees Enclosed	$549.00

XIII. METHOD OF PAYMENT OF FEES

☒ Attached is a check in the amount of $549.00.

☐ Charge Deposit Account No. **12-2475** in the amount of _____.

XIV. AUTHORIZATION TO CHARGE ADDITIONAL FEES

The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Deposit Account No. **12-2475**:

☒ 37 CFR § 1.16(a) (filing fees)

☒ 37 CFR § 1.16(b) (presentation of extra claims)

☒ 37 CFR § 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)

☒ 37 CFR § 1.17 (application processing fees)

☒ 37 CFR § 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 CFR § 1.311(b))

XV. INSTRUCTIONS AS TO OVERPAYMENT

☒ Credit Deposit Account No. **12-2475**.

☐ Refund

XVI. POWER OF ATTORNEY

☐ The power of attorney in the prior application is to _____.

☒ The power of attorney in the prior application is to Bradford J. Duft, Reg. No, 32,219.

☒ The power appears in the original papers in the prior application.

☐ The power does not appear in the original papers, but was filed on _____ in this application.

☐ A new power has been executed and is attached.

☒ Address all future communications to:

LYON & LYON LLP
633 West Fifth Street, 47th Floor
Los Angeles, California 90071
(213) 489-1600
Attention: Bradford J. Duft

XVII. MAINTENANCE OF CO-PENDENCY OF PRIOR APPLICATION

☒ A petition, fee and response has been filed to extend the term in the pending **prior** application until March 30, 1999. A copy of the petition for extension of time in the **prior** application is attached.

XVIII. CONDITIONAL PETITIONS FOR EXTENSION OF TIME IN PRIOR APPLICATION

☐ A conditional petition for extension of time is being filed in the pending **prior** application. A copy of the conditional petition for extension of time in the **prior** application is attached.

XIX. ABANDONMENT OF PRIOR APPLICATION

☐ Please abandon the prior application at a time while the prior application is pending or when the petition for extension of time or to revive in that application is granted and when this application is granted a filing date so as to make this application co-pending with said prior application. At the same time, please add the words "now abandoned" to the amendment of the specification set forth in Item 2 above.

Respectfully submitted,

LYON & LYON LLP

Dated: March 29, 1999

By: [signature]
Charles S. Berkman
Reg. No. 38,077

633 West Fifth Street, Suite 4700
Los Angeles, California 90071-2066
(213) 489-1600
Enclosures

PATENT
Attorney Docket No. 223/051

Applicant or Patentee: AMYLIN PHARMACEUTICALS, INC.
Serial or Patent No.: PCT/CA95/00287
Filed or Issued: MAY 12, 1995
For: TREATMENT OF DIABETES

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 C.F.R.1.9(f) and 1.27(c)) - SMALL BUSINESS CONCERN

I hereby declare that I am:

_____ the owner of the small business concern identified below:
__X__ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN: AMYLIN PHARMACEUTICALS, INC.
ADDRESS OF CONCERN: 9373 TOWNE CENTRE DRIVE
SAN DIEGO, CALIFORNIA 92121

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 C.F.R. 121.3-18, and reproduced in 37 C.F.R. 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of it affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full--time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third-party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the above-entitled invention described in

__X__ the specification filed herewith
____ application serial number _____, filed __
_____ Patent No. _____, issued _____

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 C.F.R. 1.9(d) or by any concern which would not qualify as a small business concern under 37 C.F.R. 1.9(d) or a nonprofit organization under 37 C.F.R. 1.9(e).

NAME _____
ADDRESS _____
_____
_____

____ INDIVIDUAL ____ SMALL BUSINESS CONCERN ____ NONPROFIT ORGANIZATION

NAME _____
ADDRESS _____
_____
_____

____ INDIVIDUAL ____ SMALL BUSINESS CONCERN ____ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to

paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small business entity is no longer appropriate. (37 C.F.R. 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so mad are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING:	Bradford J. Duft
TITLE OF PERSON OTHER THAN OWNER:	Vice President and General Counsel
ADDRESS OF PERSON SIGNING:	9373 Towne Centre Drive San Diego, California 92121

Signature _____ Date 8 Nov 96

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	:Group Art Unit:	Not Yet Assigned
JOHN DUPRE	:	
	: Examiner:	Not Yet Assigned
Serial No.: Not Yet Assigned	:	
Filed: Not Yet Assigned	:	
For: **TREATMENT OF DIABETES**	:	

PRELIMINARY AMENDMENT

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

Please amend the application as follows:

In the Claims:

Please cancel all pending claims 1-14 without prejudice. Applicant reserves the right to pursue the subject matter on this or any other appropriate patent application. The cancellation of these claims makes no admission regarding the patentability of this subject matter and should not be so construed.

SD-109113.1

CERTIFICATE OF MAILING
(37 C.F.R. §1.10)

I hereby certify that this paper (along with any referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as 'Express Mail Post Office To Addressee' in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

EL088408052US
Express Mail Label No.

March 29, 1999
Date of Deposit

Irene Grimes Betke
Name of Person Mailing Paper

Signature of Person Mailing Paper

Please add the following new claims 15-34:

15. A method of treating Type I diabetes mellitus in a mammal comprising administering to said mammal an effective amount of an insulin and an effective amount of a glucagon-like peptide 1 (7-36) amide agonist, wherein said glucagon-like peptide 1 (7-36) amide agonist is administered orally.

16. A method according to claim 15 wherein said mammal is a human.

17. A method according to claim 16 wherein said insulin and said glucagon-like peptide 1 (7-36) amide agonist are administered to the human at a selected time prior to ingestion of a meal.

18. A method according to any of claims 15-17 wherein said glucagon-like peptide 1 (7-36) amide agonist is glucagon-like peptide 1 (7-36).

19. A method according to any of claims 15-17 wherein said glucagon-like peptide 1 (7-36) amide agonist is glucagon-like peptide 1 (7-36) amide.

20. A method of treating Type I diabetes mellitus in a mammal comprising administering an effective amount of a glucagon-like peptide 1 (7-36) amide agonist, wherein said glucagon-like peptide 1 (7-36) amide agonist is administered orally.

21. A method according to claim 20 wherein said mammal is a human.

22. A method according to claim 21 wherein said glucagon-like peptide 1 (7-36) amide agonist is administered to the human at a selected time prior to ingestion of a meal.

23. A method according to any of claims 20-22 wherein said glucagon-like peptide 1 (7-36) amide agonist is glucagon-like peptide 1 (7-36).

24. A method according to any of claims 20-22 wherein said glucagon-like peptide 1 (7-36) amide agonist is glucagon-like peptide 1 (7-36) amide.

25. A method of treating Type I diabetes mellitus in a mammal comprising administering to said mammal an effective amount of an insulin and an effective amount of a glucagon-like peptide 1 (7-36) amide agonist, wherein said glucagon-like peptide 1 (7-36) amide agonist is administered nasally.

26. A method according to claim 25 wherein said mammal is a human.

27. A method according to claim 26 wherein said insulin and said glucagon-like peptide 1 (7-36) amide agonist are administered to the human at a selected time prior to ingestion of a meal.

28. A method according to any of claims 25-27 wherein said glucagon-like peptide 1 (7-36) amide agonist is glucagon-like peptide 1 (7-36).

29. A method according to any of claims 25-27 wherein said glucagon-like peptide 1 (7-36) amide agonist is glucagon-like peptide 1 (7-36) amide.

30. A method of treating Type I diabetes mellitus in a mammal comprising administering an effective amount of a glucagon-like peptide 1 (7-36) amide agonist, wherein said glucagon-like peptide 1 (7-36) amide agonist is administered nasally.

31. A method according to claim 30 wherein said mammal is a human.

32. A method according to claim 31 wherein said glucagon-like peptide 1 (7-36) amide agonist is administered to the human at a selected time prior to ingestion of a meal.

33. A method according to any of claims 30-32 wherein said glucagon-like peptide 1 (7-36) amide agonist is glucagon-like peptide 1 (7-36).

34. A method according to any of claims 30-32 wherein said glucagon-like peptide 1 (7-36) amide agonist is glucagon-like peptide 1 (7-36) amide.

REMARKS

By this Preliminary Amendment, Applicant has canceled pending claims 1-14 without prejudice and has added new claims 15-34. No new matter has been added. All new claims 15-34 find support in the original specification and claims.

CONCLUSION

For the foregoing reasons, applicants submit that the pending claims are in condition for allowance and seek an early Notice thereof. Should any issues or questions remain, the Examiner is encouraged to telephone the undersigned so that they may be promptly resolved.

Respectfully submitted,

Dated: March 29, 1999

By _____
Bradford J. Duft
Registration No. 32,219

LYON & LYON
First Interstate World Center
633 West Fifth St., Ste. 4700
Los Angeles, CA 90071-2066
(619) 552-8400

TREATMENT OF DIABETES

Field of the Invention

The present invention relates to methods and compositions for treatment of diabetes.

Background of the Invention

The recent findings of the Diabetes Control and Complications Trial (DCCT) carried out by the U.S. National Institute of Health have emphasised the importance of doing everything possible to normalise blood glucose levels in diabetics to avoid or delay micro-vascular damage. Intensified insulin therapy has been shown by the trial to improve glycaemic control but is accompanied by the risk of hypoglycaemia. This limits the degree of glycaemic control which can be safely attempted, so that true normalisation of blood glucose levels cannot be achieved with insulin therapy alone.

Glucagon-like peptide 1(7-36)amide or glucagon-like insulinotropic peptide (GLIP) is a gastrointestinal peptide which potentiates insulin release in response to glycaemia in normal humans.

Glucagon-like insulinotropic peptide has been suggested for use either alone or in conjunction with oral hypoglycaemic agents in Type II or non-insulin dependent diabetes (Gutniak et al., (1992), N.E.J.M. vol. 326, p. 1316; International Patent Application No. WO93/18786). These authors have noted a synergistic effect between the peptide and oral hypoglycaemic agents in Type II diabetics.

The present inventor has found, unexpectedly, that administration of glucagon-like insulinotropic peptide permits improved glycaemic control in subjects with insulin-requiring diabetes.

Summary of Invention

In accordance with one embodiment of the present invention, a method is provided for treating insulin-requiring diabetes in a mammal comprising administering to the mammal in a suitable regimen an effective amount of insulin and an effective amount of a peptide comprising a peptide selected from the group consisting of

(a) glucagon-like peptide 1(7-37);

(b) glucagon-like peptide 1(7-36)amide; and

(c) an effective fragment or analogue of (a) or (b).

In accordance with a further embodiment of the invention, a peptide comprising a peptide selected from the group consisting of

(a) glucagon-like peptide 1(7-37);

(b) glucagon-like peptide 1(7-36)amide; and

(c) an effective fragment or analogue of (a) or (b)

is used for the preparation of a medicament for use in the treatment of insulin-requiring diabetes in a suitable regimen which additionally comprises treatment with insulin.

In accordance with a further embodiment of the invention, a peptide comprising a peptide selected from the group consisting of

(a) glucagon-like peptide 1(7-37);

(b) glucagon-like peptide 1(7-36)amide; and

(c) an effective fragment or analogue of (a) or (b)

is used for the preparation of a medicament which also includes insulin for treatment of insulin-requiring diabetes.

In accordance with a further embodiment of the invention, a pharmaceutical composition is provided for the treatment of insulin-requiring diabetes comprising an effective amount of a peptide comprising a peptide selected from the group consisting of

(a) glucagon-like peptide 1(7-37);

(b) glucagon-like peptide 1(7-36)amide; and

(c) an effective fragment or analogue of (a) or (b) and a pharmaceutically acceptable carrier.

In accordance with a further embodiment of the invention, a method is provided for treating Type I diabetes in a mammal comprising administering to the mammal an effective amount of a peptide comprising a peptide selected from the group consisting of

(a) glucagon-like peptide 1(7-37);

(b) glucagon-like peptide 1(7-36)amide; and

(c) an effective fragment or analogue of (a) or (b).

In accordance with a further embodiment of the invention, a peptide comprising a peptide selected from the group consisting of

(a) glucagon-like peptide 1(7-37);

(b) glucagon-like peptide 1(7-36)amide; and

(c) an effective fragment or analogue of (a) or (b) is used for the preparation of a medicament for use in the treatment of Type I diabetes.

Summary of Drawings

Figure 1A shows blood levels of glucose, Figure 1B shows C-peptide, Figure 1D shows human pancreatic polypeptide (HPP), Figure 1D shows glucagon and Figure 1E shows gastrin in Type I diabetic subjects after Sustacal meal alone (○) or Sustacal meal with GLIP infusion (●).

Figure 2A shows blood levels of glucose and Figure 2B C-peptide in Type I diabetic subjects during glucose infusion alone (○) or along with IV GLIP(●).

Figure 3A shows blood levels of glucose (expressed as the change (Δ) from baseline values at time zero) and Figure 3B shows C-peptide (expressed as the change (Δ) from baseline values at time zero) in Type I diabetic subjects after Sustacal meal and saline infusion (○) or Sustacal meal with infusion of 0.75 pm GLIP/kg/min (▲).

Figure 4A shows blood levels of glucose, Figure 4B shows C-peptide, Figure 4C shows insulin and Figure 4D shows human pancreatic polypeptide (HPP) in normal subjects after Sustacal meal alone (O) or Sustacal meal immediately preceded by a subcutaneous injection of 100 μg GLIP (●).

Figure 5A shows blood levels of glucose, Figure 5B shows C-peptide, Figure 5C shows insulin and Figure 5D shows human pancreatic polypeptide (HPP) in Type I diabetic subjects after Sustacal meal alone (O) or Sustacal meal immediately preceded by a subcutaneous injection of 100 μg GLIP (●).

Figure 6A shows blood levels of glucose, Figure 6B shows C-peptide, Figure 6C shows insulin, Figure 6D shows human pancreatic polypeptide (HPP), Figure 6E shows GLIP (GLIP-1) and Figure 6F gastrin in a Type I diabetic subject who received 5 Units regular human insulin and 50 μg GLIP subcutaneously prior to a Sustacal meal.

Detailed Description of the Invention

The glucagon-like peptide 1 fragments, glucagon-like peptide 1(7-36)amide and glucagon-like peptide 1(7-37), show essentially similar insulinotropic and other biochemical effects in humans and other mammals.

Glucagon-like peptide 1(7-36)amide is referred to herein as GLIP.

The present invention provides a method of treating Type I diabetes by administration of an effective amount of GLIP, or other glucagon-like peptide 1-related peptide, either alone or in conjunction with a regimen of insulin administration.

Although the discussion herein refers to use of "GLIP", it will be understood by those skilled in the art that the therapeutic methods of the invention may be practised by employing GLIP, glucagon-like peptide 1(7-37), an effectiye peptide including GLIP or glucagon-like peptide 1(7-37), or an effective fragment or analogue of GLIP or glucagon-like peptide 1(7-37).

As is seen in Figure 2, IV administration of GLIP along with intravenous glucose stimulated secretion of endogenous insulin in the subjects studied and gave improved control of blood glucose level. These subjects were in the remission phase, or so-called "honeymoon phase", of IDDM characterised by substantial remaining endogenous insulin secretion.

The same dose of GLIP (1.2 pm/kg/min) gave excellent control of blood glucose level in these subjects after a meal, as seen in Figure 1, Panel A. Under these conditions, GLIP infusion also prevented a significant increase in blood levels of C-peptide.

After the Sustacal meal, the test subjects showed normal secretion of pancreatic polypeptide (PP) but this response was absent during GLIP infusion (Figure 1, Panel C). It is believed that this abrogation of PP response was due to the delayed passage of the meal from the stomach to the small intestine as a result of GLIP administration. That it was not due to a general suppression of gastrointestinal peptide secretion is indicated by the normal gastrin response to the presence of food in the stomach in these subjects (Figure 1, Panel E).

Administration of GLIP prevented the mean rise in plasma glucagon levels stimulated by the meal in the absence of GLIP. Gastrin levels were not significantly affected.

Administration of a lower dose of GLIP (0.75 pmol/kg/min) along with a meal resulted in some increase in blood glucose and C-peptide, as seen in Figure 3. Although the increase in glucose was less than in the control experiment, the rise in C-peptide was similar to the control experiment.

GLIP is known to cause delay of gastric emptying in humans and other mammals (Wettergren et al., (1993), Digestive Diseases and Sciences, v. 38, p. 665). As seen in Figure 4, when GLIP is given subcutaneously to normal

subjects prior to ingestion of a meal, there is a delay of 30 to 60 minutes in the rise in blood glucose level. This delay is likely due to inhibition of gastric emptying.

When Type I diabetics were given GLIP subcutaneously prior to ingestion of a test meal, a lowering of blood glucose levels was seen compared to the control figures when no GLIP was administered (Figure 5, Panel A). The delayed rise in pancreatic polypeptide (HPP) levels (Panel D) indicate delayed gastric emptying. As seen from Panels B and C, there was no enhancement of insulin secretion over control levels to account for the lower glucose levels.

It may be that the improved glycaemic control seen with GLIP administration in Type I diabetics is due to delay of the post-meal rise in blood glucose through the interval required for the establishment of the effect of insulin.

The efficacy of GLIP administration along with insulin in restraining the expected rise in blood glucose after a standard meal in Type I diabetes is seen in Example 6 and Figure 6. 50 μg GLIP was administered along with half the insulin dose that would usually be required to deal with the test meal. As seen in Figure 6, Panel A, blood glucose did not rise above 8 mM. With this size of meal and half the usual insulin dose, considerably higher blood glucose levels would have been expected, in the absence of the effect of GLIP. For example, with this meal and no insulin, blood glucose levels reached 15 mM, as seen in Figure 5, Panel A.

As seen from Figure 6, Panel E, GLIP was cleared from the blood in about two hours so that pre-meal GLIP administration would not be expected to interfere with management of subsequent meals.

When GLIP is used to improve glycaemic control in Type I diabetics having residual endogenous insulin secretion capacity, both the insulinotropic effect of the

hormone and its effect to delay gastric emptying will contribute to its effect. Some remission phase Type I subjects may be sufficiently controlled by administration of GLIP alone, without exogenous insulin.

In the majority of patients with Type I diabetes, however, treatment with a regimen including both GLIP and insulin is likely to be required. These studies indicate that the observed effects of GLIP on glycaemia are not dependent on stimulation of insulin release and are therefore not limited to diabetics retaining residual insulin secreting capacity.

The use of GLIP in treating Type I diabetes, in accordance with the present invention, provides improved glycaemic control and reduces post-prandial excursions of blood glucose. This accords with the current emphasis on normalising blood glucose levels as much as possible, to reduce diabetic complications.

Furthermore, a regimen combining administration of insulin and administration of GLIP, in accordance with the present invention, is applicable to patients with insulin requiring diabetes which would not strictly be classified as Type I.

An insulin-requiring diabetic is a diabetic who is unable to avoid hyperglycaemia without the use of insulin. The invention further provides a method for treating patients with diabetes which is etiologically Type II but requires insulin treatment.

Diabetics frequently find the requirements for food intake and insulin administration at midday particularly irksome and an interference with work and other activities. By administering GLIP to diabetic subjects at breakfast time, along with administration of longer acting insulin if necessary, diabetics may be able to omit lunch or greatly reduce the size of that meal, and thereby avoid the need for midday insulin.

The delayed adsorption of nutrients when both GLIP and insulin are administered before breakfast will also reduce the risk of hypoglycaemia if lunch is delayed.

The studies described herein also indicate that a therapeutic regimen including both GLIP and insulin will in many cases permit the use of reduced doses of insulin. This is also beneficial in the avoidance of hypoglycaemia.

GLIP or its related peptides which may be employed in the treatment methods described herein may be administered orally, nasally or parenterally. Parenteral administration may be by a variety of routes including subcutaneous or intravenous infusion, and subcutaneous or intravenous injection.

The regimen of GLIP or GLIP and insulin administration required to give the desired glycaemic control in a diabetic patient can be readily determined by those skilled in the management of diabetic patients.

As will be understood by those skilled in the art, any suitable insulin preparation may be used in conjunction with GLIP administration in the combined regimen described herein.

Suitable insulins include regular or fast-acting insulin to maintain blood glucose control through the post-prandial interval, with or without addition of longer-acting insulin to maintain blood glucose control through the post-absorptive interval.

The dosages of GLIP required may be optimised for each subject by evaluation of the degree of glycaemic control achieved by trial doses.

Another convenient method of monitoring the level of effect of GLIP on a subject is to monitor the blood level of pancreatic polypeptide in response to trial doses of GLIP.

Such dosage and regimen adjustments are now commonplace, for example for diabetics treated with mixtures of fast and slow acting insulins. These mixed

preparations are available in various ratios of fast to slow and an appropriate ratio must be selected for a particular patient by trial doses. One patient may even employ insulin preparations of different ratios to handle varying sizes of meals. By similar testing, a suitable GLIP and insulin regimen may be selected.

GLIP and insulin may be administered separately or may be prepared and administered as a single formulation.

EXAMPLES

Example 1

7 subjects with remission phase Type I diabetes were studied after ingestion of a standardised meal of Sustacal (Upjohn) (delivering 30 kg/kg). Subjects continued their normal insulin treatment programme on the day prior to the test and, on the day of the test, omitted their morning insulin injection and arrived fasting at 8:00 am. On one test day they were given the Sustacal meal, followed immediately by initiation of intravenous infusion of GLIP (synthetic human GLIP-(7-36)amide from Peninsula, U.K.) at an infusion rate of 1.2 pm/kg/min. Infusion was continued for 120 minutes. Blood levels of glucose, C-peptide, gastrin, glucagon and HPP were monitored by standard radioimmunoassay methods in samples taken before and at intervals during the study, up to 180 minutes. On another test day, subjects were given the Sustacal meal alone and the same analytes were similarly monitored.

Results are shown in Figure 1.

Example 2

Four subjects with remission phase Type I diabetes were studied during intravenous glucose infusion. Subjects prepared for the tests as described in Example 1, but received an intravenous infusion of glucose (20 g

over 60 min. constant rate) instead of the Sustacal meal. On one test day, they also received intravenous GLIP for 60 minutes (1.2 pm/kg/min for 60 min.) and on another test day, they received IV glucose alone. Blood levels of glucose and C-peptide were monitored as in Example 1.

The results are shown in Figure 2.

Example 3

Four subjects with remission phase Type I diabetes were studied during infusion with 0.75 pm/kg/min GLIP for 120 minutes after a Sustacal meal.

The test was conducted as described in Example 1 and blood glucose and C-peptide levels were measured. On a further test day, the subjects were studied during saline infusion after a similar Sustacal meal.

Results are shown in Figure 3.

Example 4

7 normal volunteers were studied after ingestion of a Sustacal meal either alone or immediately preceded by a subcutaneous injection of 100 μg GLIP.

Results are shown in Figure 4. *indicates statistically significant differences between treatments ($p<0.05$).

A delay in increase in blood levels of glucose, HPP, C-peptide and insulin was seen. When the experiment was repeated with 50 μg or 200 μg dose of GLIP, proportionally shorter and longer delays, respectively, were seen.

Example 5

7 Type I diabetic subjects were studied. Subjects omitted their morning insulin injection on the days of the tests and were given a Sustacal meal alone one day and, on another day, a Sustacal meal immediately preceded by a subcutaneous injection of 100 μg GLIP.

The results are shown in Figure 5. *indicates statistically significant differences between treatments ($p<0.05$).

Example 6

One Type 1 diabetic subject was given GLIP along with insulin and the effects on post-prandial glycaemia observed. The subject received 5 units of insulin and 50 μg GLIP as subcutaneous injections immediately prior to ingestion of a Sustacal meal as described in Example 1. The results are shown in Figure 6. Blood levels of GLIP were monitored by a standard radioimmunoassay method.

Although only preferred embodiments of the present invention have been described, the present invention is not limited to the features of these embodiments, but includes all variations and modifications within the scope of the claims.

I CLAIM:

1. A method of treating insulin-requiring diabetes in a mammal comprising administering to the mammal in a suitable regimen an effective amount of insulin and an effective amount of a peptide comprising a peptide selected from the group consisting of

(a) glucagon-like peptide 1(7-37);

(b) glucagon-like peptide 1(7-36)amide; and

(c) an effective fragment or analogue of (a) or (b).

2. The method of claim 1 wherein the mammal is a human.

3. The method of claim 2 wherein an effective amount of insulin and an effective amount of a peptide comprising a peptide selected from the group consisting of

(a) glucagon-like peptide 1(7-37);

(b) glucagon-like peptide 1(7-36)amide; and

(c) an effective fragment or analogue of (a) or (b) are administered to the human at a selected time prior to ingestion of a meal.

4. The method of any of claims 1 to 3 wherein the insulin-requiring diabetes is Type I diabetes.

5. The method of any of claims 1 to 3 wherein the insulin-requiring diabetes is Type II diabetes.

6. Use of a peptide comprising a peptide selected from the group consisting of

(a) glucagon-like peptide 1(7-37);

(b) glucagon-like peptide 1(7-36)amide; and

(c) an effective fragment or analogue of (a) or (b) for the preparation of a medicament for use in the treatment of insulin-requiring diabetes in a suitable

regimen which additionally comprises treatment with insulin.

7. Use of a peptide comprising a peptide selected from the group consisting of

(a) glucagon-like peptide 1(7-37);

(b) glucagon-like peptide 1(7-36)amide; and

(c) an effective fragment or analogue of (a) or (b) for the preparation of a medicament which also includes insulin for treatment of insulin-requiring diabetes.

8. Use of a peptide in accordance with claim 6 wherein the insulin-requiring diabetes is Type I diabetes.

9. Use of a peptide in accordance with claim 7 wherein the insulin-requiring diabetes is Type I diabetes.

10. A pharmaceutical composition for the treatment of insulin-requiring diabetes comprising an effective amount of a peptide comprising a peptide selected from the group consisting of

(a) glucagon-like peptide 1(7-37);

(b) glucagon-like peptide 1(7-36)amide; and

(c) an effective fragment or analogue of (a) or (b) and a pharmaceutically acceptable carrier.

11. A pharmaceutical composition in accordance with claim 10 for the treatment of Type I diabetes.

12. The pharmaceutical composition of claim 10 or 11 further comprising an effective amount of insulin.

13. A method of treating Type I diabetes in a mammal comprising administering to the mammal an effective amount of a peptide comprising a peptide selected from the group consisting of

(a) glucagon-like peptide 1(7-37);

(b) glucagon-like peptide 1(7-36)amide; and

(c) an effective fragment or analogue of (a) or (b).

14. Use of a peptide comprising a peptide selected from the group consisting of

(a) glucagon-like peptide 1(7-37);

(b) glucagon-like peptide 1(7-36)amide; and

(c) an effective fragment or analogue of (a) or (b) for the preparation of a medicament for use in the treatment of Type I diabetes.

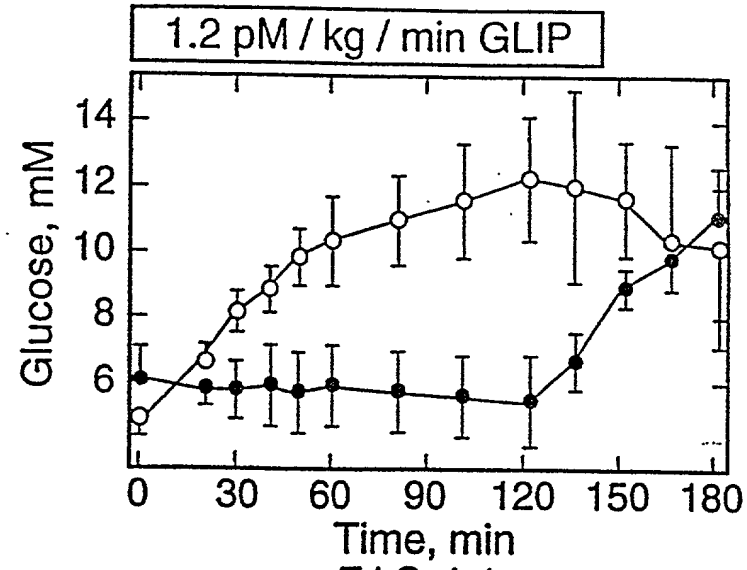

FIG.1A.

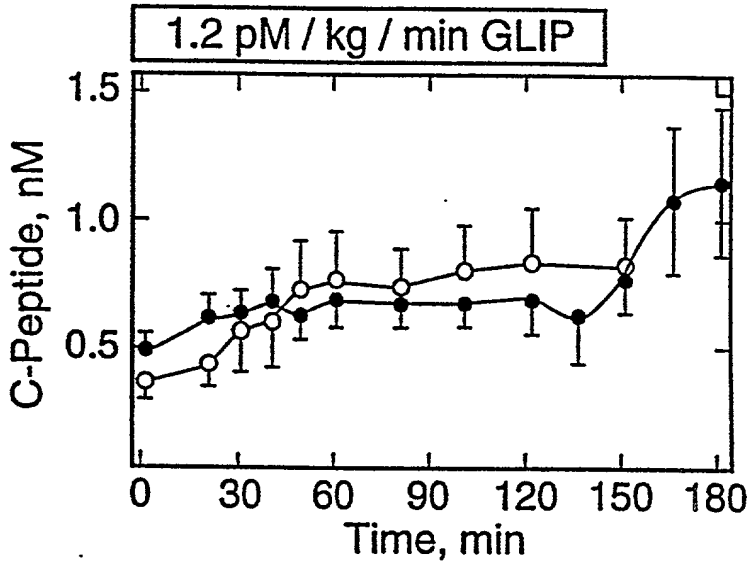

FIG.1B.

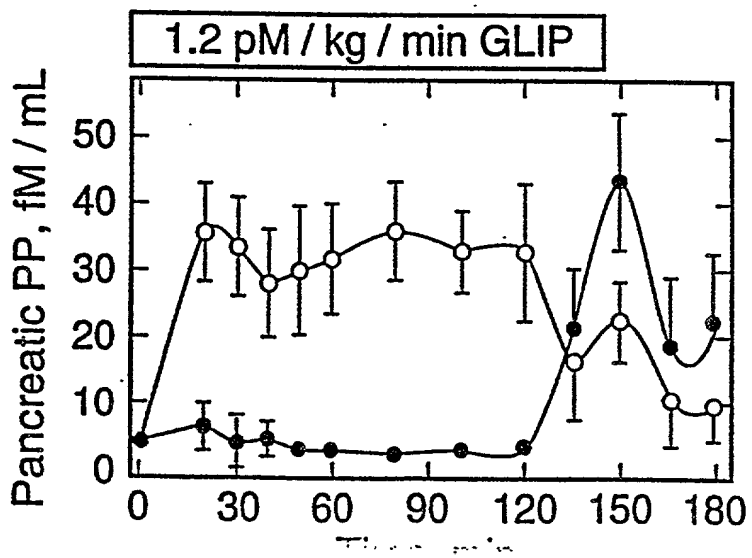

FIG.1C.

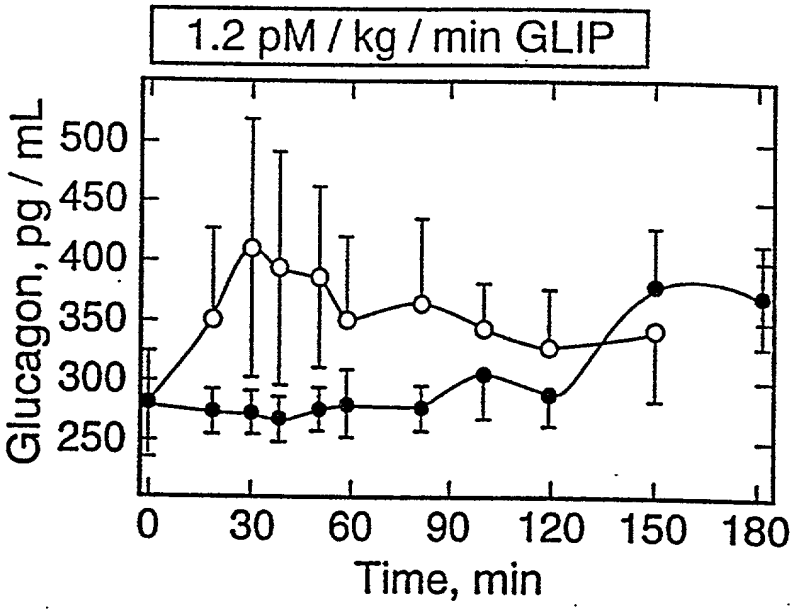
1.2 pM / kg / min GLIP
Glucagon, pg / mL
500
450
400
350
300
250
0
30
60
90
120
150
180
Time, min

FIG.1D.

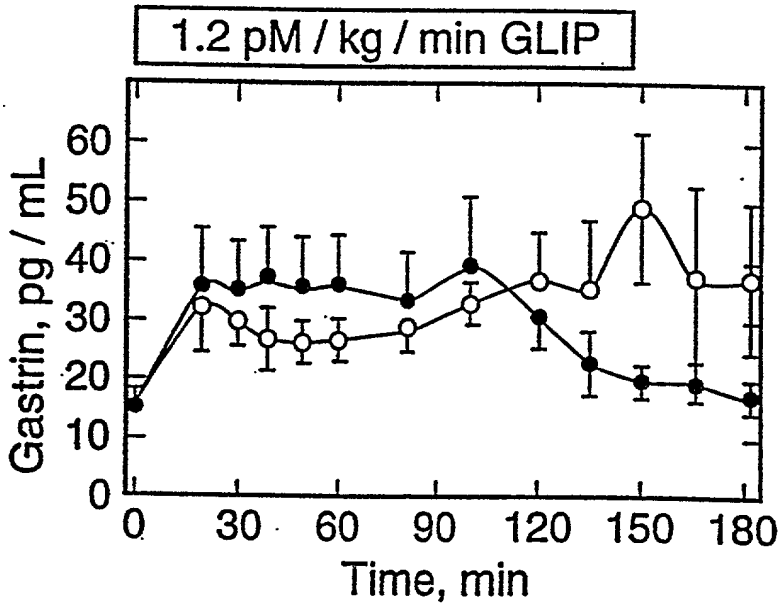
1.2 pM / kg / min GLIP
Gastrin, pg / mL
60
50
40
30
20
10
0
0
30
60
90
120
150
180
Time, min

FIG.1E.

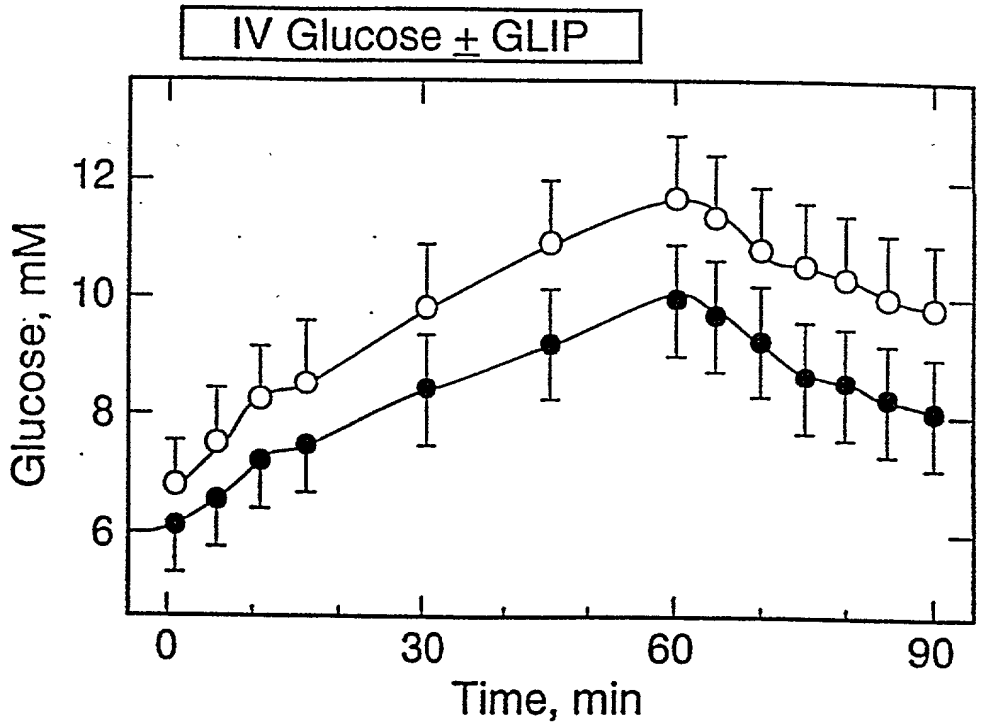
IV Glucose ± GLIP
Glucose, mM
12
10
8
6
0
30
60
90
Time, min

FIG.2A.

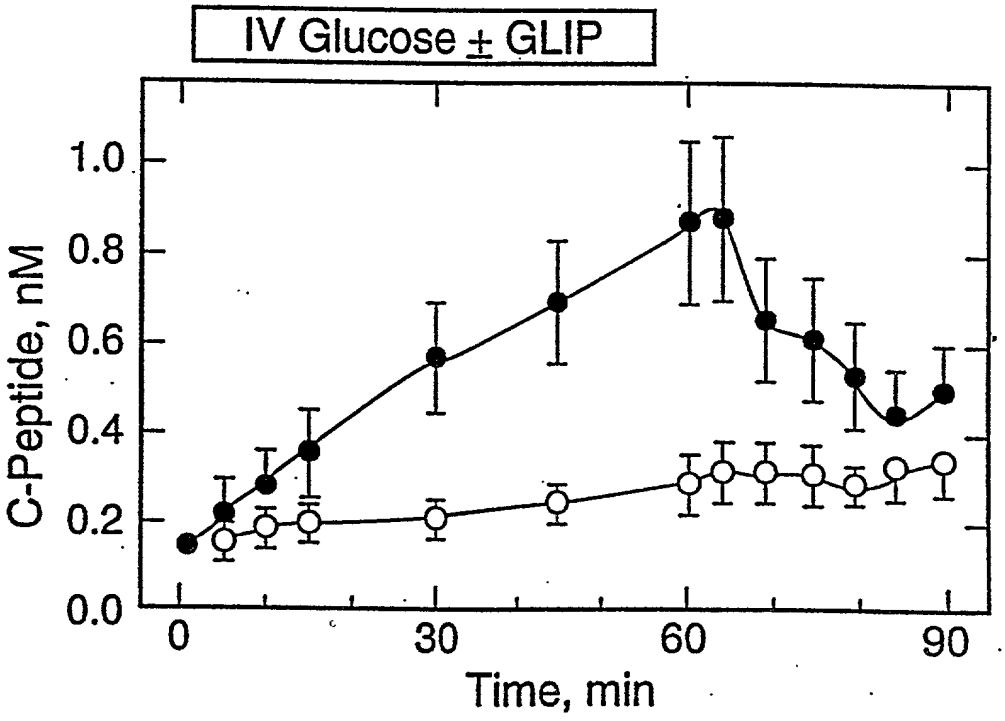
IV Glucose ± GLIP
C-Peptide, nM
1.0
0.8
0.6
0.4
0.2
0.0
0
30
60
90
Time, min

FIG.2B.

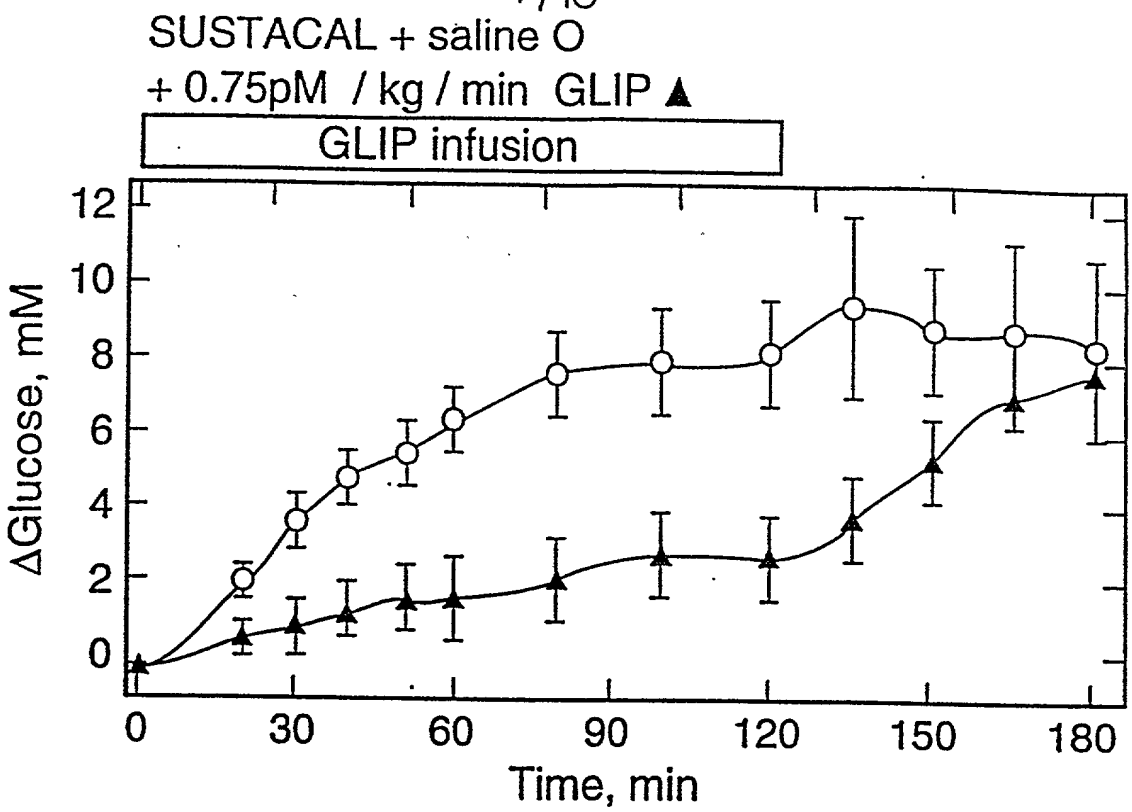
SUSTACAL + saline O
+ 0.75pM / kg / min GLIP ▲
GLIP infusion
ΔGlucose, mM
12
10
8
6
4
2
0
0
30
60
90
120
150
180
Time, min

FIG. 3 A.

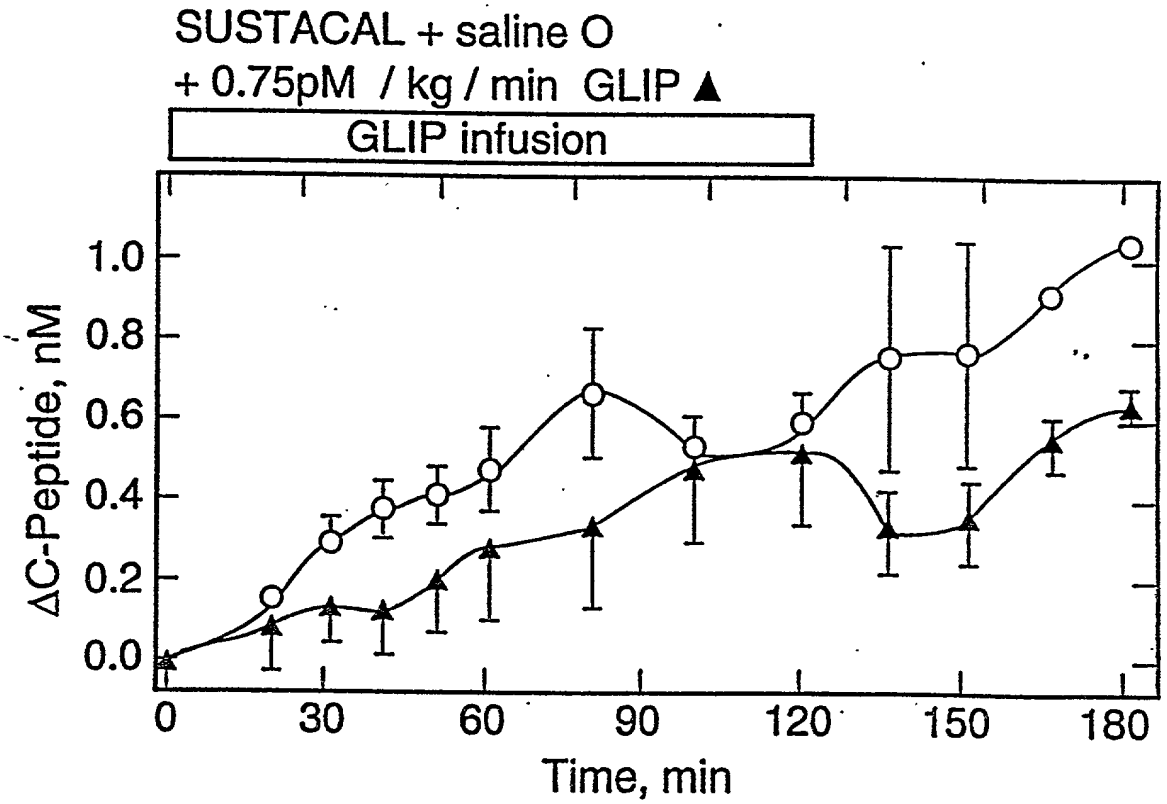
SUSTACAL + saline O
+ 0.75pM / kg / min GLIP ▲
GLIP infusion
ΔC-Peptide, nM
1.0
0.8
0.6
0.4
0.2
0.0
0
30
60
90
120
150
180
Time, min

FIG. 3 B.

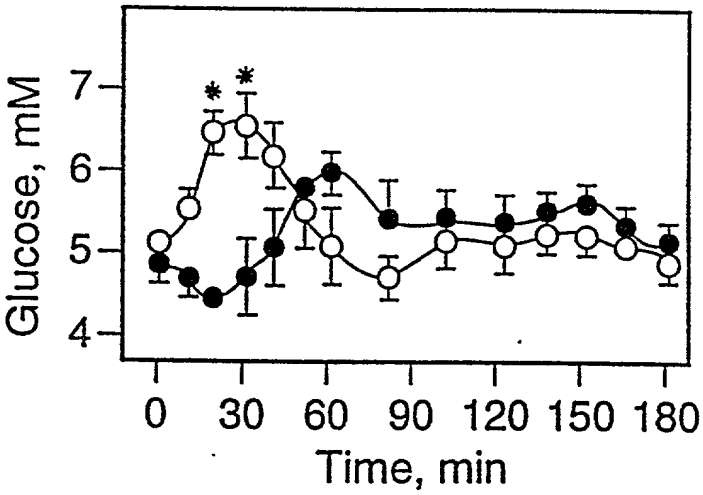
Glucose, mM
7
6
5
4
*
*
0
30
60
90
120
150
180
Time, min

FIG.4A.

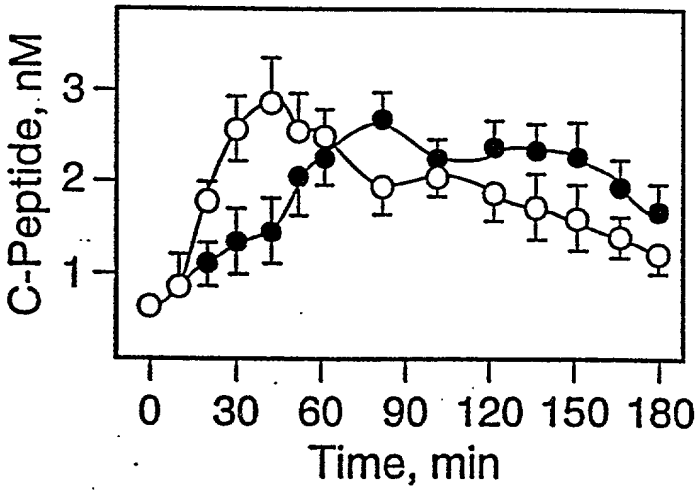
C-Peptide, nM
3
2
1
0
30
60
90
120
150
180
Time, min

FIG.4B.

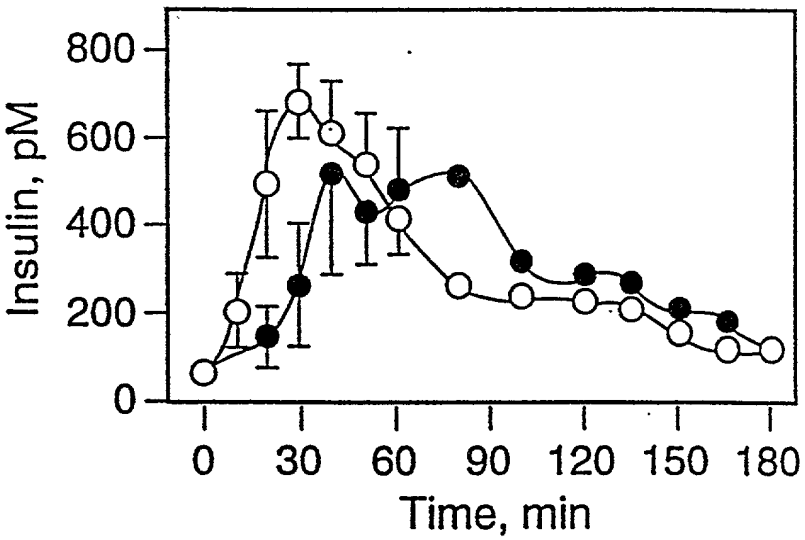
800
600
400
200
0
Insulin, pM
0
30
60
90
120
150
180
Time, min

FIG.4C.

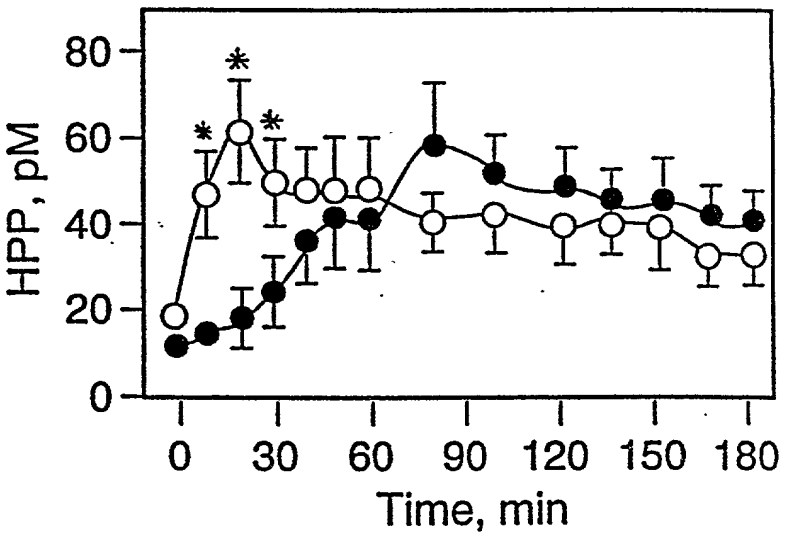
80
60
40
20
0
HPP, pM
*
*
*
0
30
60
90
120
150
180
Time, min

FIG.4D.

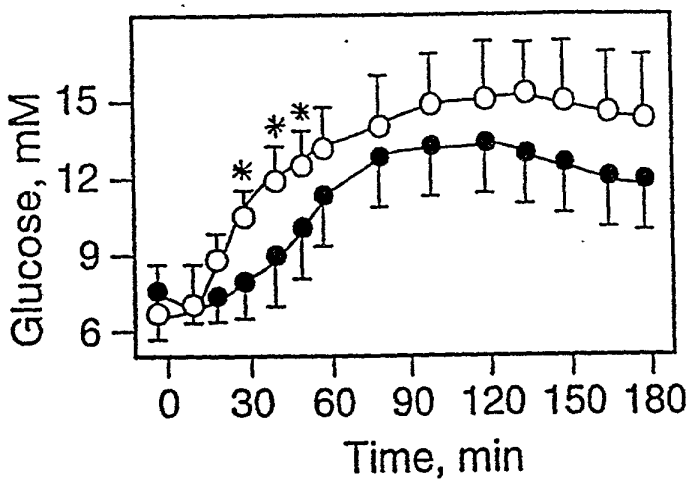
Glucose, mM
15
12
9
6
0
30
60
90
120
150
180
Time, min
*
*
*

FIG.5A.

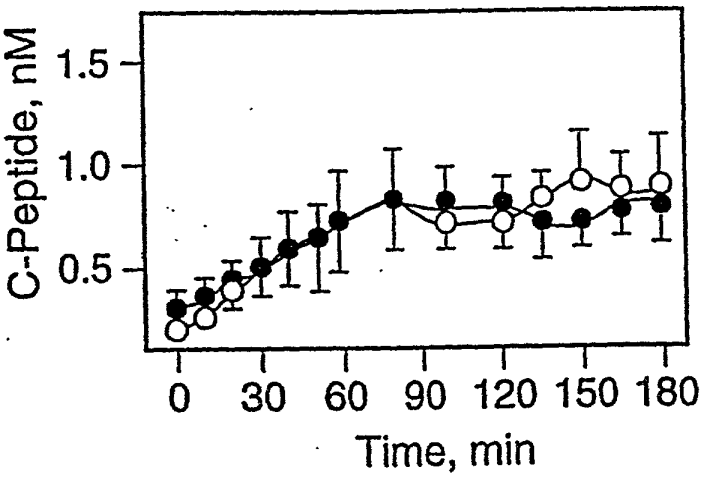
C-Peptide, nM
1.5
1.0
0.5
0
30
60
90
120
150
180
Time, min

FIG.5B.

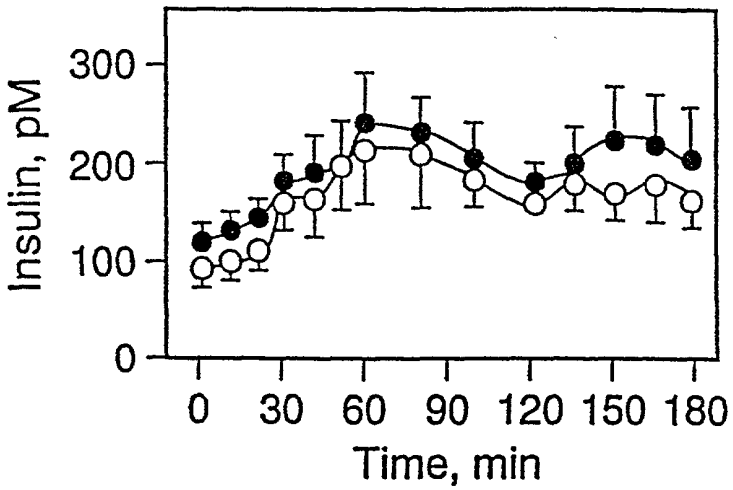
Insulin, pM
300
200
100
0
0 30 60 90 120 150 180
Time, min

FIG.5C.

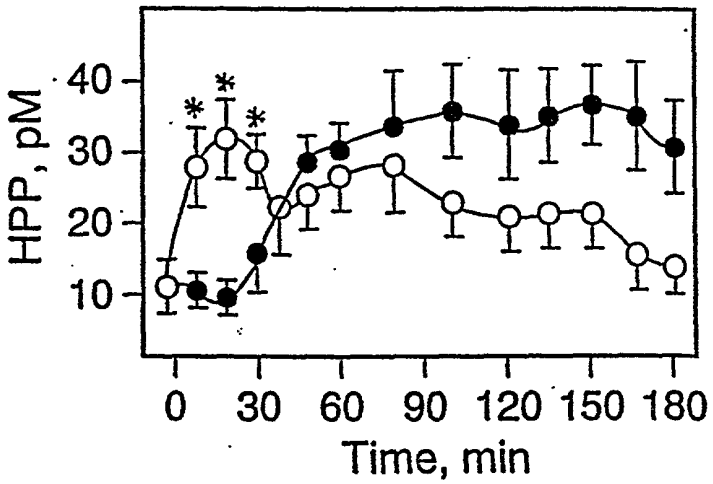
HPP, pM
40
30
20
10
*
*
*
0 30 60 90 120 150 180
Time, min

FIG.5D.

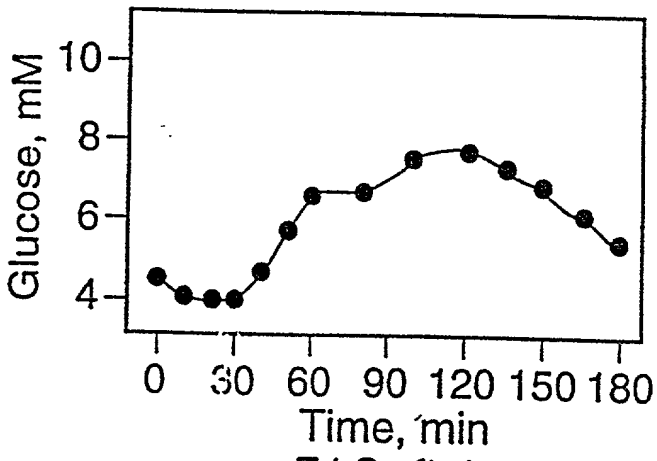

FIG.6A.

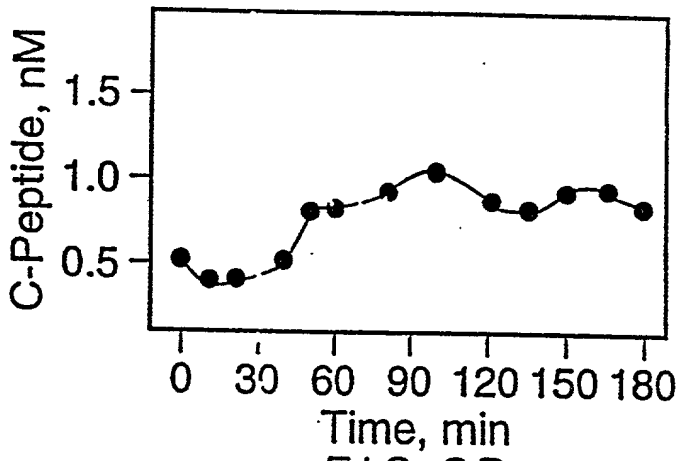

FIG.6B.

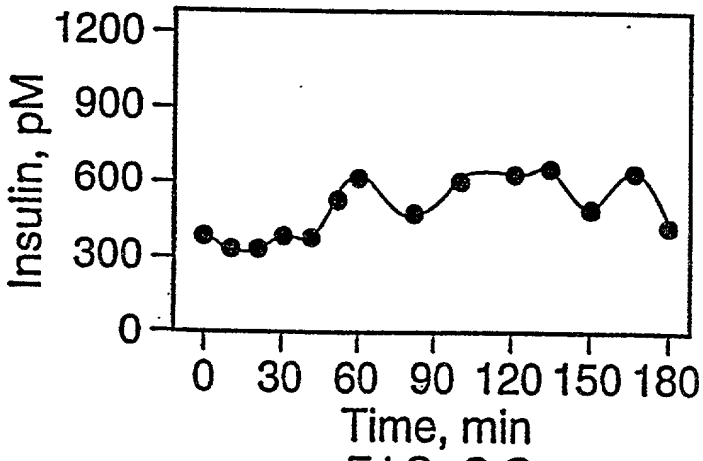

FIG.6C.

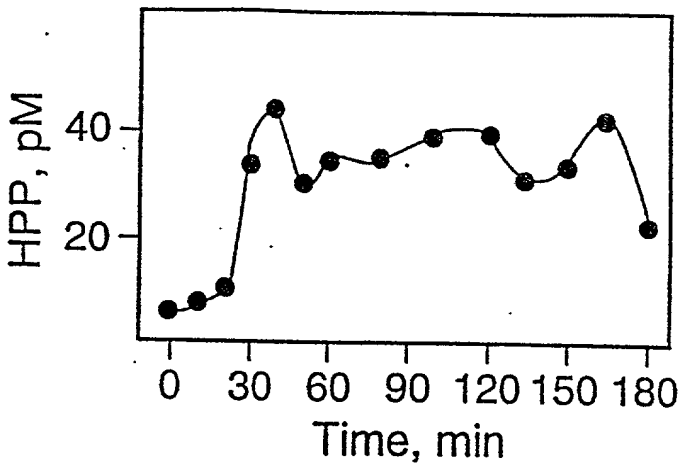

FIG.6D.

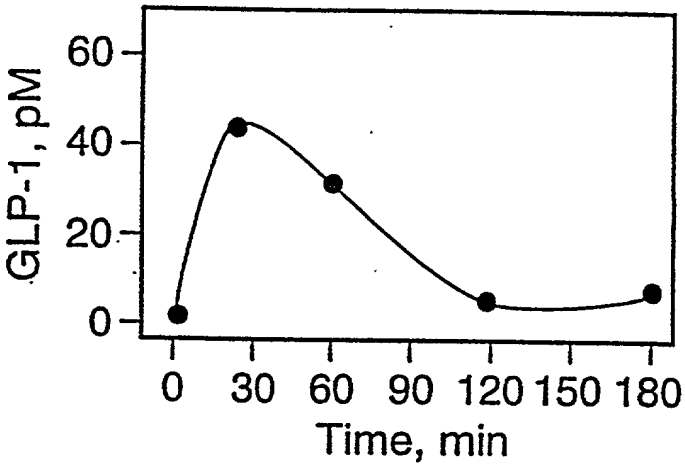

FIG.6E.

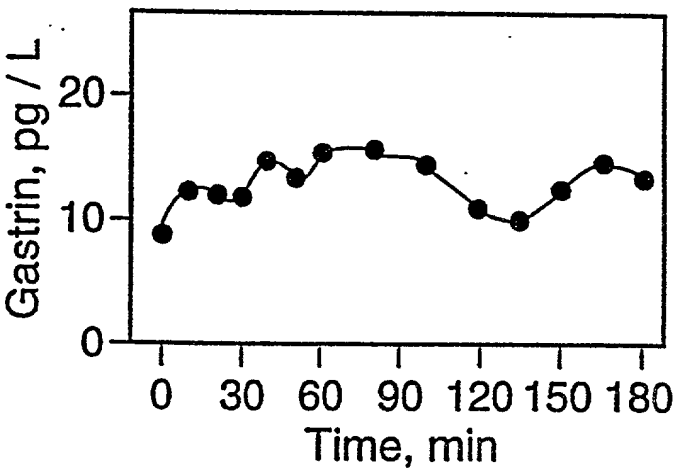

FIG.6F.

U.S. DEPARTMENT OF COMMERCE — FORM 13-11 — 13-116.5

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY
(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER: 223/051

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

TREATMENT OF DIABETES

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application

Serial No. _____

on _____.

and was amended

on _____ (if applicable).

☒ was filed as PCT international application

Number PCT/CA95/00287

on 12 May 1995.

and was amended under PCT Article 19

on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (IF PCT, INDICATE "PCT")	APPLICATION NUMBER	DATE OF FILING (DAY, MONTH, YEAR)	PRIORITY CLAIMED UNDER 35 USC 119
GB	940946.8	12.05.94	☒ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO

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(Combined Declaration For Patent Application and Power of Attorney—PTO 1391 [13-11]—page 1 of 2)

Combined Declaration For Patent Application and Power of Attorney (Continued)

ATTORNEY'S DOCKET NUMBER: 223/051

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS		STATUS (Check one)		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE	U.S. SERIAL NUMBERS ASSIGNED (IF ANY)			
PCT/CA95/00287	12 May 1995			XX	

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (List name and registration number)

Bradford J. Duft, Reg. No. 32,219

Send Correspondence to:
Bradford J. Duft
Lyon & Lyon LLP
633 West Fifth Street, Los Angeles CA 90071

Direct Telephone Calls to: (name and telephone number)
213-489-1600

101	FULL NAME OF INVENTOR	FAMILY NAME: DUPRE	FIRST GIVEN NAME: John	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY: London	STATE OR FOREIGN COUNTRY: Ontario, Canada	COUNTRY OF CITIZENSHIP: Canadian
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: 72 Sherwood Ave	CITY: London, Ontario	STATE & ZIP CODE/COUNTRY: Canada N6A 2G2
202	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY
203	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon

SIGNATURE OF INVENTOR 101	SIGNATURE OF INVENTOR 202	SIGNATURE OF INVENTOR 203
John Dupre		
DATE: Jan 8 1997	DATE	DATE

PTO 1391 (REV. 8-92) Page 2 of 2 — U.S. DEPARTMENT OF COMMERCE Patent and Trademark Office

(Combined Declaration For Patent Application and Power of Attorney—PTO 1391 [13-11]—page 2 of 2)